On the Cytomorphosis of the Enamel Organ in the Hake.

By

J. Thornton Carter,

Department of Zoology, University of London, University College.

With Plates 22, 23 and 24.

In the Gadidæ each tooth is surmounted by a pointed cap of enamel which rests on a platform of dentine, whose central area extends into the enamel cap, thus affording a firm support without increasing the outside dimensions of the tooth over this area. The development of this enamel cap is associated with certain marked changes in the enamel cells investing it.

So far as I am aware there is but one published paper dealing with the formation of enamel in the Gadidæ—"Upon the Development of the Enamel in Certain Osseous Fish," C. S. Tomes, 'Phil. Trans.,' cxciii, B. 186, 1900—and the cytological conclusions advanced therein were so much at variance with the appearances found in a number of fishes and other vertebrates in which I had followed out a complete cytomorphosis of the enamel cells, and also with isolated sections of developing teeth of Gadidæ in my possession that I have worked out the full life-history of the cells forming the enamel cap in the Hake (Merluccius vulgaris), as this was the creature principally employed by Tomes.

Through the kindness of Prof. Meek one or two heads were obtained at sea and immediately fixed in corrosive-formalin-acetic mixture. In one of these heads the fixation is very

fine and from it about fifty teeth and developing tooth-germs have been isolated, cut into complete series of sections of 10 μ, and stained with iron hæmatoxylin, followed by a counter-stain. Serial sections are a necessity when endeavouring to obtain a complete cytomorphosis where it is imperative to know the exact plane of a section for comparison with other stages.

The origin of the tooth-germ is as in Mammals, there being an ingrowth of the deeper layer of the oral epithelium and the growth of a dentine papilla which becomes invested by the epithelium except at its base.

The epithelial enamel organ consists of two layers of cells, the one lying in apposition to the dentine, consisting of columnar ameloblasts with well-defined cell outlines and the nuclei lying about the centres of the cells (Pl. 22, fig. 1, *a*.). Immediately external, separating the ameloblasts from the surrounding connective tissues lies a layer of polygonal cells usually two or three deep, constituting the external epithelium of the enamel organ (*e.e.*). Throughout the whole life-history of the enamel organ these two layers of cells remain in contact, there being no such differentiation as is seen in Mammals, where the ameloblasts and external epithelium become separated by the modified cells known as the stellate reticulum, which act as a storehouse for the materials to be elaborated by the ameloblasts into the secretion which gives origin to the enamel. The absence of such provision is associated with a marked modification of the ameloblasts.

With the appearance of a very thin layer of dentine over the apex of the dentine papilla the columnar ameloblasts undergo a marked change (Pl. 22, fig. 2). Their nuclei are seen to have receded somewhat towards the bases of the cells, the cytoplasm about them still preserving the outlines of the individual cells, but between the nuclei and the secreting surface—i. e. toward the dentine—the individual outlines of the cells are lost owing to a very rapid formation of metaplasm in this area which lies in vacuoles (*vac.*) separated by a fibrillar cytomitoplasm (*c.m.t.*) whose fibrils run fairly parallel

to the long axis of the cell. The contents of these vacuoles, when not washed away, appear faintly coagulated and tinged with the counter-stain, whilst the fibrils of the cytomitoplasm are basophile.

This process of vacuolation or accumulation of metaplasm progresses towards the cell base and is associated with a considerable increase in the length of the cells (Pl. 22, figs. 3 and 4). The nuclei become considerably elongated and are situate at various levels in different cells, more frequently lying somewhat towards the secreting surface. At this stage a considerable amount of ameloblastic secretion has been deposited on the surface of the dentine (Pl. 22, fig. 6, *a s.*), and there is no sign of any merging of the cells into the secretion, such as one would find did the ameloblasts themselves become transformed into a stroma which became incorporated into the enamel. Though the outlines of the individual cells are lost, the secreting surface presents a well-defined regular continuous margin, a sharp line in longitudinal section, and a finely granular lamina in oblique section, in strong contrast to the vacuolated cytoplasm (Pl. 22, figs. 4, 5, 6, *i.a.m.*). This secreting surface of the cell, the inner ameloblastic membrane, is not a metaplasmic product but is a specialised area of the cytoplasm, showing traces of a fine polygonal structure corresponding to individual cell areas, and the fibrils of the cytomitoplasm appear to terminate in certain granules lying immediately below the surface. When this stage is reached, the shedding of the ameloblastic secretion is very active, leading to a marked diminution in the size of the vacuoles and decrease in their number (Pl. 22, fig. 5), and this passage of the contents of the vacuoles carries with it the great majority of the nuclei until they appear to rest almost in contact with the inner surface of the inner ameloblastic membrane. The fibrils of the cytomitoplasm (*c.m.t.*) now become arranged in more or less straight lines running parallel with the long axes of the enamel cells.

When this stage in enamel formation is reached there is usually a considerable amount of dentine formed below the

shoulder on which the enamel cap rests. This dentine also is invested by elongated enamel cells (Pl. 23, fig. 8), which, however, do not increase in length very greatly, and do not undergo the changes which take place in those cells corresponding to the area occupied by the enamel cap. They are usually separated from the cells of the external epithelium by a sharply defined line, and the portion of their cytoplasm between this line and the nuclei appears almost clear, whilst the surface of the nuclei towards it is concave. This is probably due to some metabolic activity, for with the complete formation of the enamel cap and of the dentine of the body of the tooth this appearance of the cell is lost, the nucleus becomes (Pl. 23, fig. 9) of a rounded oval form, and the cytoplasm throughout the whole cell becomes faintly granular.

Except for the enamel cap, I do not think any enamel is formed over the surface of the tooth, for all teeth composed entirely of vaso-dentine exhibit a considerable degree of flexibility, and with carefully ground sections of fresh teeth, I have not been able to demonstrate the existence of even the thinnest layer of any highly refractile substance, the dentine ending sharply in a well-defined margin, as seen in Pl. 23, fig. 9, at *d*.

When the tooth has erupted and become functional these cells rapidly lose their columnar form and assume a flattened polygonal shape.

The restoration of the outlines of individual cells foreshadowed at the stage shown in Pl. 22, fig. 5, becomes well marked in the next (Pl. 22, fig. 7), where the distance between cell base and secreting surface has diminished greatly, the nuclei (*n*.), now deeply chromatic and much elongated, have receded towards the centres of their respective cells, and the individuality of each cell has again become apparent. This recovery of the cell usually progresses until the nuclei have become of an oval shape and the chromatin aggregated into two or three masses (Pl. 23, fig. 10, *a*.).

The formation of the enamel cap now appears to be complete, since if germs at this stage of development are

dissected out, the transparent caps of enamel are seen resting on their shoulders of dentine. The enamel appears to be fully calcified, and may be ground down into very thin sections without disclosing any trace of structure, but on washing such a section with an acid, a faint prismatic structure is revealed.

With the full development of the enamel cap the process of eruption of the tooth proceeds rapidly, and is accompanied by certain marked changes in the enamel cells, whose function has now been accomplished. An ingrowth of the cytoplasm of certain cells of the external epithelium takes place which, inserting itself between the ameloblasts, divides them up into groups or nests, frequently with no definite arrangement of the latter, but often causing them to become arranged in a series of loops as seen in Pl. 23, fig. 10, where the ameloblasts (*a.*) are seen arranged regularly about these ingrowths of the external epithelium (*e.e.*), whilst the inner ameloblastic membrane (*i.a.m.*) preserves an even contour towards the formed enamel. In all my preparations of germs which had completed the formation of the enamel cap, the cells of the external epithelium invade the ameloblasts, and this invasion is most marked along a line corresponding to that area where the enamel cap will emerge in eruption. When the apex of the tooth is to be the point first to emerge, the cells of the external epithelium insert themselves between the bases of the ameloblasts over the whole vertex of the enamel organ forming a deep furrow. Sometimes there are two furrows running almost parallel, as in Pl. 23, fig. 12 (*e.e.*), and the ameloblasts (*a.*) lying beneath become changed, so that their nuclei stain with difficulty, and their cytoplasm disappears in a sort of coarse vacuolation.

The apex of the tooth, however, is not invariably the point first to emerge from its investing epithelium, and when the tooth is going to erupt sideways the cells of the external epithelium (*e.e.*) traverse a small sector opposite which the rarefaction of the ameloblasts proceeds to permit of its exit (Pl. 23, fig. 11).

Associated with these regressive changes there is an

ingrowth of the oral epithelium, which on reaching the enamel organ appears to extend about it for some distance, inserting itself between the enamel cells and the tissues of the tooth sac. This is particularly well seen in Pl. 23, fig. 14, where the ingrowth of the cells (*o.e.*) extends down to the very limits of the cells responsible for the enamel cap. In Pl. 24, fig. 15, is shown a somewhat greater magnification of a similar condition in which it is seen that the ingrowth where close to the tooth-germ consists in section of a double row of cells (*o.e.*).

This ingrowth rapidly increases in size until it forms a large mass of epithelial cells (Pl. 24, fig. 16, *o.e.*), in contact at one end with the enamel organ over the enamel cap of the tooth and at the other end continuous with the cells of the oral mucous membrane. In Pl. 24, fig. 17, is shown a transverse section through such an ingrowth taken about midway between the tooth-germ and the surface. It is seen to be merely a mass of epithelial cells with no sign of any lumen or other evidences of glandular structure and seems to be solely a provision for the eruption of the tooth similar to the ingrowth of oral epithelium which Profs. Wilson and Hill have shown to obtain in Marsupials.

The cells overlying the formed enamel cap now consist of groups or nests of elongated ameloblasts interspersed with cells of the external epithelium (Pl. 24, fig. 16). With the emergence of the tooth the columnar form of the ameloblasts is rapidly lost (Pl. 24, fig. 18, *a.*) and they become rounded cells undergoing degenerative changes which lead to their rapid disappearance (Pl. 24, fig. 19). The epithelial cells which invest the remainder of the tooth and which were continuous with the ameloblasts forming the enamel cap (Pl. 23, figs. 8 and 9) rapidly lose their columnar form so that the erupted functional tooth is invested by a layer of flattened polygonal cells.

These changes which I have described are so much at variance with the appearances described by Tomes in the paper mentioned at the commencement of this paper that it is necessary to quote from it at some length.

He writes:

"In its earliest stages the tooth-germ of the Hake does not present any marked peculiarities and resembles a mammalian tooth-germ . . . there is an enamel organ which consists of the usual double row of cells, the inner of which (ameloblasts) form the internal epithelium of the enamel organ and are elongated columnar cells 19 μ in length.

"At the next stage which it is necessary to describe, a very thin skin of dentine has calcified. . . . The space above the top and sides of the dentine germ is occupied by a delicate tissue which has a reticulated appearance and reaches quite out to the walls of the tooth sac, thus occupying the position of an enamel organ. But in it none of the usual constituents of an enamel organ can be recognised; there are no ameloblasts, no stellate reticulum, nor external epithelium of the enamel organ, but in their place and in the position but a short time before occupied by the ameloblasts is this reticulated stroma. . . .

"This stroma has a general appearance of fibrillation in a direction at right angles to the dentine surface . . . its outermost portion always stains much more deeply than the rest and rounded forms are there seen which at first I was inclined to regard as nuclei, the nuclei of the transformed ameloblast cells. But in sections which lie at right angles to the long axis of the tooth-germ . . . it is found that though the stained areas are circular, their outer borders are indefinite, and that they surround sharply-defined circular areas which are less deeply or not at all stained; this seems to negative the idea of the stained areas seen in longitudinal section being really nuclei. . . .

"A cross section some distance within the stroma shows nothing but circular areas, lying separated from one another and with a delicately striated tissue intervening between them. The rings vary from 3 μ to 5·5 μ in diameter. . . . When the section is oblique the rings become ovals, sometimes much elongated, so that they appear to be sections of either rods or tubes of considerable length. . . .

"So far the stroma has been shown to consist of two elements, the sharply-defined tubes or rods and a delicately fibrillated tissue which intervenes between them.

"To revert to the rods or tubes . . . it seems hardly possible to doubt that they bear some relation to the forming prisms and are a stage in their development, and the fact that they are not to be distinguished in that portion of the enamel stroma which lies close to the dentine tends to bear out this view.

"Two facts are perfectly clear; the first, that the enamel of these fishes is certainly not an excretion from the ends of the ameloblasts, for they have disappeared long before calcification takes place; the other, that the calcification does take place in the form of a conversion of or a deposition in a pre-existent stroma of definite arrangement."

I have quoted at some length from Tomes since he has employed his conclusions in elaborating a theory of enamel formation which supposes fundamentally different processes in different groups of Vertebrates. Little comment is necessary. The bodies which he was at first inclined to regard as the nuclei of ameloblasts are the cells of the external epithelium (Pl. 23, fig. 13, *e.e.* and *n.*), whilst his "rods or tubes" are the nuclei of the ameloblasts or the vacuoles.

Since this paper was written, Mr. J. H. Mummery has published a paper "On the Structure and Development of the Tubular Enamel of the Sparidæ and Labridæ" ('Phil. Trans.,' B. ccclix, vol. 208), in which he describes certain modifications leading to a definite "conversion of the whole enamel organ into a system of glands and blood-vessels."

His interpretations are based largely on the views of Tomes, quoted above, and he concurs that "the enamel organ is converted into a stroma traversed by tube-like prolongations," the transformed ameloblasts being incorporated into the enamel. With the disappearance of the ameloblasts their function is assumed by an ingrowth of glandular tissue "derived from the deep layer of the submucous tissue of the mouth and pharynx (the italics are mine), which separates "the lime salts from the circulating blood to form the calcified

enamel, the organic matrix of which is formed by the transformed ameloblasts, which have become converted into the stroma."

In a succeeding paragraph Mummery speaks of "these invasions of the enamel organ by prolongations of glandular tissue from the deep surface of the epithelium of the mouth" and "of their direct communication with the unmistakable glandular tissue of the mouth." Thus he would seem to indicate some such ingrowth of the oral epithelium as I have shown to take place after the formation of the enamel cap in the Hake, and which I believe to be connected with the eruption of the tooth.

Certainly, though in Hake the enamel organ of a growing tooth-germ is richly invested by capillaries lying in the surrounding connective tissues there is no sign in my preparations of any vascularity of the enamel organ or of the epithelial ingrowth, nor any evidence of this latter structure having a secretory function.

In Sargus, Mummery writes, "that part of the enamel near the dentine is laid down by true ameloblast cells, the rest of the enamel being formed after these cells have disappeared and the tubes and stroma have taken their place." . . . "From the evolutionary standpoint this seems a very puzzling problem," which it certainly is.

Mummery's figures disclose no cytological detail, but, in so far as his conclusions are based on concurrence with Tomes' deductions from the enamel organ of the Hake, they are negatived by the evidence I have advanced in this communication.[1]

Since the ameloblasts maintain their individuality throughout the whole period of the formation of the enamel cap, it follows that the fully-developed enamel is the product of certain changes taking place in the ameloblastic secretion, which at first occupied the vacuoles in the ameloblasts, and then passed through the inner ameloblastic membrane to be deposited on the surface of the dentine. The substance occupying the vacuoles is clear, taking the acid stain faintly;

[1] See addendum.

the inner ameloblastic membrane persists throughout the whole life-history of these cells, but the ameloblastic secretion having passed through the membrane, is intensely basophile (Pl. 22, fig. 6, *a.s.*). The deposition of this secretion commences immediately after the formation of the first thin layer of dentine and prior to the vacuolation of the ameloblastic layer to form the appearances seen in Pl. 22, figs. 2, 3, and 4, it then goes on continuously and rapidly, so that the fully calcified cap is completed long before the tooth attains its full size and becomes functional.

When but a small amount of ameloblastic secretion has been deposited on the surface of the dentine, as in Pl. 24, fig. 20 (*e*), it forms a sharply-pointed cap, which is easily distorted in shape and easily detached from the dentine. This cap exhibits a spongiform structure in its central area (Pl. 24, fig. 21), the meshes being basophile; towards the periphery the staining takes place much more deeply, and the structure is much more regular, in many areas presenting a regular honeycomb skeleton of organic matrix.

Since these changes take place in a transparent fluid secretion and an organic framework first appears, which eventually undergoes solidification, with the final assumption of a prismatic crystalline structure, it seems reasonable to advance the interpretation that the changes leading to the ultimate solidification and complete calcification of the products of the activity of the ameloblasts are purely physico-chemical changes similar to those known to be passed through by other colloids in their solidification, e.g. a coagulation or gel-formation of the secretion first takes place, the structure of the gel depending on the nature of the protein in the secretion; this precipitation of a large amount of the organic material causes a greater concentration of the lime salts in the fluid occupying the interspaces, and calcification then progresses until the organic matrix is almost, if not entirely, calcified.

Twenty years ago Prof. G. C. Bourne, in his paper "On the Calcareous Skeleton of the Anthozoa," wrote:

"We are ignorant of the laws which govern the formation of these organic crystalline growths as we are of the molecular laws which determine why a given mineral solution shall crystallise out according to a given system."

Much work remains to be done, but to-day, owing to the great advances in our knowledge of colloidal chemistry, we are no longer in complete ignorance of these laws, and with regard to enamel we are in a position to protest yet more strongly "against the discovery of a Deus ex machina in the form of calcified cells."

My thanks are due to Mr. Pittock, of the Department of Zoology, University College, for his kind assistance in the preparation of material, and to Miss M. Rhodes, of the Lister Institute, for the drawings used in illustration of this paper.

Addendum.

I have had no opportunity of examining the genera employed by Dr. Mummery, but, since the proofs of this paper have been in my hands, I have looked over a complete series of sections of the jaws and pharyngeal plates of Pagellus centrodontus and of Labrus bergylta, which I took at sea this autumn and fixed immediately.

My material lends no support to the views put forward by Mummery, for, though the enamel organ becomes richly vascular, the vessels assuming the arrangement figured by him, in place of his "glands with distinct central ducts" and his "tubes," I find that the ameloblasts persist throughout the whole period of enamel formation, separated from the capillaries—which have well-defined endothelial walls—by the cells of the external epithelium. Further, the cytoplasm near the forming enamel becomes modified to constitute the inner ameloblastic membrane, the interspaces between contiguous cells being closed by the development of a structure which, so to speak, cements these areas together, and gives to a section a pattern of well-defined polygonal outlines, each polygon corresponding to an individual ameloblast.

EXPLANATION OF PLATES 22, 23 and 24,

Illustrating Mr. J. Thornton Carter's paper "On the Cytomorphosis of the Enamel Organ in the Hake."

[All the drawings were made direct by means of the Abbe camera lucida.]

The following is a list of the reference letters common to the various figures: *a.* Ameloblasts. *a.s.* Ameloblastic secretion. *c.m.t.* Cytomitoplasm. *d.* Dentine. *d.p.* Dentine papilla. *e.* Enamel. *ee.* External epithelium. *i.a.m.* Inner ameloblastic membrane. *n.* Nucleus. *o.e.* Oral epithelium. *vac.* Vacuoles.

PLATE 22.

Fig. 1.—Longitudinal section through a tooth-germ prior to the formation of the shoulder of dentine on which the base of the enamel cap will rest. The ameloblasts (*a.*) present a columnar form and the outlines of individual cells are quite distinct. × 70.

Fig. 2.—Portion of a longitudinal section through the enamel organ. The outlines of the individual cells have disappeared in the area between the nuclei (*n.*) and the secreting surface (*i.a.m.*), though still visible in the area between the bases of the cells and about the nuclei. × 450.

Fig. 3.—Portion of a longitudinal section through the enamel organ. The outlines of individual ameloblasts have now disappeared due to the formation of vacuoles (*vac.*) in the cytoplasm and the nuclei lie at various levels. × 450.

Fig. 4.—Transverse section through the enamel organ showing the vacuolation of the cytoplasm, the vacuoles (*vac.*) being separated by a cytomitoplasm (*c.m.t.*). Towards the secreting surface the vacuolation disappears and there is a layer, finely granular in structure, in which may be discerned the faint outlines of the ends of the individual ameloblasts. × 450.

Fig. 5.—Portion of a longitudinal section through the enamel organ. The vacuoles seen in the preceding figure are now disappearing owing to the passage of their contents through the inner ameloblastic membrane (*i.a.m.*) and the nuclei (*a.*) have been carried down almost to the secreting surface. The restoration of the outlines of the individual ameloblasts is faintly foreshadowed. × 450.

Fig. 6.—Transverse section through a tooth-germ showing the central core of dentine (*d.*) surrounded by the forming enamel (*a.s.*), beyond which lie the ameloblasts (*a.*) invested by the cells of the external epithelium (*e.e.*). × 70.

Fig. 7.—Longitudinal section through a portion of the enamel organ in which the outlines of individual ameloblasts have become visible again, all trace of vacuolation having disappeared. The nuclei no longer lie at the extreme ends of the cells but are distributed at various levels. × 450.

PLATE 23.

Fig. 8.—Longitudinal section through a portion of the enamel organ showing the transition of the cells responsible for the enamel cap (seen at the stage described in fig. 5.) into the cells which invest the dentine of the body of the tooth. × 300.

Fig. 9.—Longitudinal section through a portion of the enamel organ at a point lower than the enamel cap where no enamel is formed and corresponding to the shorter cells (*a.*) figured in the preceding illustration. A portion of the enamel cap (*e.*) is seen, also fragments of dentine (*d.*) × 400.

Fig 10.—Longitudinal section of the enamel organ showing the ameloblasts (*a.*) arranged in a series of loops with the cells of the external epithelium (*e.e.*) inserting their cytoplasm between these loops. × 250.

Fig. 11.—Transverse section through the enamel organ showing the cells of the external epithelium (*e.e.*) inserting themselves between the ameloblasts (*a.*) and thus forming a furrow. × 300.

Fig. 12.—Longitudinal section of a portion of the enamel organ from over the apex of a tooth showing the cytoplasm of certain cells of the external epithelium (*e.e.*) extending inwards and the ameloblasts beneath undergoing rarefaction. × 450.

Fig. 13.—Transverse section through a portion of the enamel organ stained to show the outlines of the cells of the external epithelium (*e.e.*). The outlines of the ameloblasts are not visible, and the drawing is not continued inwards sufficiently far to show their nuclei. × 450.

Fig. 14.—Longitudinal section through a tooth-germ showing an ingrowth of the oral epithelium (*o.e.*), which, passing over the apex of the tooth extends down to its base, inserting itself between the external epithelium and the surrounding connective tissues. × 50.

PLATE 24.

Fig. 15.—Longitudinal section through the apex of a tooth-germ showing the top of the enamel cap (*e.*) with its investing ameloblasts (*a.*). An ingrowth of the oral epithelium (*o.e.*) is seen which in close proximity to the tooth-germ appears in the drawing as a double row of cells. × 200.

Fig. 16.—Longitudinal section through the enamel organ in the area of the cap. The ameloblasts (*a.*) are seen becoming separated into groups or nests owing to the ingrowth of the cells of the external epithelium (*e.e.*). Extending from the surface into contact with the external epithelium is an ingrowth of the oral epithelium (*o.e.*). × 80.

Fig. 17.—Transverse section through an ingrowth of the oral epithelium such as shown at *o.e.* in the preceding figure. The ingrowth is seen to present an irregular outline corresponding to extensions of the investing connective tissues. × 250.

Fig. 18.—Longitudinal section through the apex of a tooth which has just emerged through the overlying tissues, showing the dentine (*d.*) with its shoulder and central core to afford support to the enamel cap which is partly dissolved, its tip being seen at *e*. There is no trace of the columnar ameloblasts seen in preceding figures, but their site is occupied by a mass of rounded cells (*a.*) continuous with the flattened cells investing the dentine below the shoulder. × 70.

Fig. 19.—A portion of the area (*a.*) of the preceding figure showing the rounded cells, which are the original ameloblasts undergoing cytolytic changes. × 450.

Fig. 20.—Longitudinal section through a developing tooth showing the first formed layer of dentine (*d.*) surmounted by the forming enamel cap (*e.*). × 120.

Fig. 21.—A portion of the developing enamel seen in the preceding figure showing its spongiform structure. × 450.